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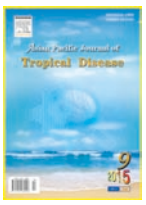

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Characterization of drug resistance mutations in ART-naïve HIV-1 infected children in Northern Vietnam

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ABSTRACT

Objective: To investigate the profile of drug resistance-associated mutations in pol gene of antiretroviral therapy-naïve HIV-1 infected children enrolled in National Hospital Pediatrics in Northern Vietnam.

Methods: Genotyping was performed on 134 antiretroviral therapy-naïve plasma samples from HIV-1 infected children. HIV-1 pol gene was amplified using primers for protease and reverse transcriptase and sequenced using the BigDye chemistry. The mutations were analyzed based on the Stanford University HIV-1 Drug Resistance Database and ISA-USA list.

Results: All the children were infected with HIV-1 CRF01_AE subtype. Major protease inhibitor resistance mutations were found in 2 children (2.3%) and reverse-transcriptase inhibitor resistance mutations were found in 5 children (7.7%). The protease inhibitor mutations were observed M46L and L90M and reverse-transcriptase inhibitor mutations were M184I, K65R, Q151M, T69N, L210W, Y181C, M230L and K101E.

Conclusions: This is the first study reporting the prevalence of drug resistance-associated mutation in naïve HIV-1 infected children in Northern Vietnam. These data also emphasize the importance of genotypic resistance testing of HIV-1 infected children before initiating treatment in order to achieve better clinical outcome.

1. Introduction

HIV-1 infected children still remain a significant public health problem in Vietnam. The reported number of HIV-1 infected children continues to increase from 2 727 in 2005 to 4 719 in 2009 and 5 700 in 2012[1]. The wide spread use of combination antiretroviral therapy (cART) for the treatment of HIV-1 infected children has dramatically changed the course of HIV-1 infection, leading to reduction in morbidity and mortality among the HIV-1 infected population[1-4]. In Vietnam, antiretroviral therapy has been intensively introduced since 2005 by the Vietnamese government, resulting in an increase in cART coverage of HIV-1 infected children from 15.7% in 2005, 49.7% in 2009 and 82% by the end of 2011[2,3]. However, together with increasing number of patients on antiretroviral therapy, there is also an increased probability of viruses developing both drug resistance mutations (DRMs) and transmitting these mutant viruses.

The prevalence of DRM was less than 5% in 2006, 1.7% for protease inhibitor (PI) and 4.5% for reverse-transcriptase inhibitor (RTI) in 2008 and 7.6% RTI in 2009 among ART-naïve adult patients in Northern Vietnam[5-9]. DRM rate of 7.6% has been reported by a study carried out in HIV-1 infected drug-naïve children in Ho Chi Minh City (HCMC) in the southern area[10]. DRM rate among people failing multiple lines of cART was similar across study population in HCMC, ranging from 49% to 55% in adults and 50% in children (aged 0-14 years)[4].

The purpose of this analysis was to investigate the profile of

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drug resistance-associated mutation in pol gene of ART-naïve HIV-1 infected children enrolled in National Hospital Pediatric in Northern Vietnam.

2. Materials and methods

2.1. Study population

One hundred and thirty-four HIV-1 infected children were selected from 500 of children cared at National Hospital of Pediatric (NHP) in Northern Vietnam between December 2009 and December 2011. HIV-1 infected children come from 21 out of 29 provinces in Northern Vietnam. Mean age of the children was 50 months (ranging from 1 to 144 months). Ethical clearance was obtained from the National Hospital of Pediatric Ethics Committee and an informed consent form and questionnaire were provided to each parents or guardians of the patients for acceptance prior to participation in the study.

2.2. Samples and laboratory techniques

Ethylene diamine tetraacetic acid blood samples were corrected before they start cART. Plasma was separated and stocked at -80 ℃ till analysis. Viral RNA was extracted from 140 μL of HIV-1 positive plasma using a commercial kit (QIAamp Viral RNA Mini Kit, QIAGEN Inc., Valencia, CA), according to the manufacturer's instructions. The HIV-1 pol gene protease (PR) and reverse transcriptase (RT) regions were amplified by one-step RT-PCR and nested PCR using AmpliTaq Gold (Applied Biosystems, Japan) and/or KOD FX (Toyobo, Osaka, Japan). The PR region was amplified by nested RT-PCR with primers DRPRO5 (5'-AGACAGGYTAATTTTTTAGGGA-3') and DRPRO2L (5'-TATGGATTTTCAGGCCCAATTTTTGA-3') in the first round and DRPRO1M (5'-AGAGCCAACAGCCCCACCAG-3') and DRPRO6 (5'-ACTTTTGGGCCATCCATTCC-3') in the second round as previous[6,7], and the RT region was amplified by nested RT-PCR with primers DRRT1L (5'-ATGATAGGGGGAATTGGAGGTTT-3') and Rtout (5'-ATATACTCCATGCACAGGGGTTTT-3') in the first round, and DRRT7L (5'-GACCTACACCTGTCAACATAATTGG-3') and DRRT6L (5'-TAATCCCTGCATAAATCTGACTTGC-3') in the second round[6,7].

RT-PCR was done with one cycle at 55 ℃ for 30 min and one cycle at 94 ℃ for 2 min, then 40 cycles at 94 ℃ for 15 s, 55 ℃ for 30 s, and 68 ℃ for 1 min, with a final extension of 68 ℃ for 5 min[6,7]. Nested PCR for pol-PR was done with one cycle at 95 ℃ for 10 min, followed by 40 cycles at 95 ℃ for 30 s, 55 ℃ for 30 s, and 72 ℃ for 1 min, with a final extension at 72 ℃ for 10 min. Nested PCR for pol-RT was done with one cycle at 94 ℃ for 1 min, and 35 cycles at 98 ℃ for 10 s, 55 ℃ for 30 s and 68 ℃ for 5 min[6,7]. The amplicons were electrophoresed on 1.5% agarose gel and visualized under UV light after staining with ethidium bromide. Nucleotide sequencing was performed using ABI Prism 3130 auto sequencer and followed by editing with Genetyx software version 9.1.

2.3. DRM and phylogenetic analysis

The presence of DRMs in the pol-PR and pol-RT regions was detected using the Stanford University HIV Drug Resistance Database (http://hivdb.stanford.edu/) and the International AIDS Society-USA Spring 2013 Mutation List[11,12]. Phylogenetic analysis was performed using genotype CRF01_AE subtype reference sequences from the GenBank and Los Alamos Databases with HIV-1 genotype B (HBX2) as the out group. The neighbor-joining method with 1 000 bootstrap replicates was used and bootstrap value more than 700 was considered as significant.

2.4. Sequence data

GenBank accession numbers of the sequences reported in this study were KJ541962-KJ542012 for pol-PR/RT, KJ542013-KJ542046 for pol-PR and KJ542047-KJ542063 for pol-RT.

3. Results

3.1. Characteristics of patients

One hundred and thirty-four HIV-1 positive children were included in the study (45.5% were male and 54.5% were female). The mean age was 50 months (ranging from 1 to 144 months). The median CD4 cell count was 476 cells/mm^3 (range: 40-1 697 cells/mm^3). The median viral load was 990 300 copies/mL (range: 1 603-28 373 494 copies/mL).

3.2. DRMs in ART-naïve patients

We analyzed 134 ART-naïve HIV-1 infected children samples; 86 and 65 were successful analyzed in the pol PR and pol RT regions, respectively (Table 1). Two (2.3%) of 86 pol PR sequences had major PI resistance-associated mutations, one with single mutation at M46L (DR 92) and another with multi-resistance mutations with M46L and L90M (DR 01). Of the 65 pol-RT sequences analyzed, five (7.7%) had major RTI resistance-associated mutations: two with nucleoside RTIs (NRTIs) resistance-associated mutations M184I (DR 35), T69N and L210W (DR 190), and two with non-NRTIs (NNRTIs) resistance-associated mutations, Y181C (DR 84 and DR 172), and M230L (DR 154). One with multiple DRMs was in both regions: K65R, Q151M in NRTIs and Y181C, K101E in NNRTIs (DR 172).

Table 1

Amino acid substitutions associated with DRM in ART-naïve patients.

Sample ID	Age (year)/ Gender	HIV-1 subtype	PI resistance mutations	RTI resistance mutations	
				NRTIs	NNRTIs
DR 01	1.25/Female	CRF01_AE	M46L, L90M		
DR 35	6/Male	CRF01_AE		M184I	
DR 84	2/Male	CRF01_AE			Y181C
DR 92	2/Female	CRF01_AE	M46L		
DR 154	5/Female	CRF01_AE			M230L
DR 172	4/Male	CRF01_AE		K65R, Q151M	K101E, Y181C
DR 190	4/Male	CRF01_AE		T69N, L210W	

3.3. Phylogenetic analysis

HIV-1 genotypic analysis based on pol sequences showed that all the children were infected with CRF01_AE subtype. Most of the CRF01_AE strains isolated from NHP Vietnam formed a large cluster with the reference sequences from Guangxi, Southern China, and Northern Vietnam, while some were related to strains from HCMC, Cambodia, and/or Thailand (data not shown).

4. Discussion

World Health Organization Global Resistance Network recommends that HIV drug resistance surveillance focus on newly HIV diagnosed individuals in countries where treatment access is being expanded[13]. Although, data on primary resistance in Vietnamese adults is available, there is few information regarding to the pediatrics population in Northern Vietnam. The results of the present study might facilitate the tracking of trends in the transmission of resistance locally.

The prevalence of PI DRM's was 2.3% and the prevalence of NRTI DRM's was 7.7% in our ART-naïve HIV-1 infected populations between 2009 and 2011. The prevalence of PI DRM was slightly higher than the result in previous study in 2009 (1.7%) in ART-naïve HIV-1 infected adult patients in Northern Vietnam[6], and the prevalence of NRTI was similar with the previous result reported in studies carried out in drug-naïve children in Southern Vietnam (7.6%)[10].

This finding could indicate that the DRM rate among ART-naïve HIV-1 infected children was not significantly different in the northern area and the southern area where the individuals with higher income started cART earlier and initially with single or dual drug regimens. Our result (7.7%) was also similar with the previous result (7.6%) in ART-naïve HIV-1 infected adult in Northern Vietnam in 2009[5]. This is the first report on the DRM rate in ART-naïve HIV-1 infected Vietnamese children in Northern Vietnam.

PI mutations M46L and L90M were observed in two children; these mutations result in reduced susceptibility to commonly used lopinavir/ritonavir. RTI DRMs (M184I and Y181C) were detected in two HIV-1 infected children. These mutations are common in Vietnamese patients and have been reported in previous studies[5-8]. In previous study, we also reported that the Y181C mutation was detected in two-thirds of the cases among HIV-infected children on ART[14], and 25% (2/8) of children, who exposed with single-dose nevirapine during the prevention of mother-to-child transmission[15]. The M184I mutation causes resistance to lamivudine (3TC) and emtricitabine (FTC), both of which are NRTIs. This mutation causes high-level resistance to nevirapine, 2-fold decreased susceptibility to efavirenz, and 5-fold decreased susceptibility to etravirine and rilpivirine[5].

Q151M and K65R substitutions, and insertions at codon 69 in the reverse transcriptase encoding region of HIV genome confer resistance to a large range of NRTIs and are thus called multi-nucleoside resistance (MNR) mutations[16-19]. In Vietnam, the frequency of Q151M and K65R were reported from 4% to 9% in adults and children in the south but it was not reported previously in north of Vietnam[4,20,21]. In the current study, MNR mutations K65R and Q151M were detected in one child. This is the first report of Q151M in Northern Vietnam. We also found the MNR (T69N) and thymidine analog mutation (L210W) mutations in one child. The T69 mutation is not a common mutation in Vietnam and has not reported in previous studies[5-8,22-25]. The HIV-1 DRMs detected in our population may have been primary or transmitted from mothers harboring primary or transmitted DRMs. Selection of mutation by d4T drastically limits the choice of antiretroviral molecules available for second-line therapy in resource-limited settings.

There were some limitations in our study: first, the sample size of HIV-1 drug-naïve children was small, which means that the estimated proportion of children with drug resistance might be an underestimate; second, we didn't have a maternal history on cART and mother-to-child prophylaxis.

The prevalence of antiretroviral DRM among ART-naïve children was 10.0% (2.3% for PI and 7.7% for RTI), which is slightly higher than the prevalence reported in the previous studies in ART-naïve adult patients in Northern Vietnam. This result suggests that continuous HIV-1 characterization and evaluation of DRM before therapy would be important in guiding patients' directed treatment.

Conflict of interest statement

The authors declare no conflict of interest.

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References

[1] National Committee for AIDS, Drugs, and Prostitution Prevention and Control. Viet Nam AIDS response progress report 2012. Vietnam: National Committee for AIDS, Drugs, and Prostitution Prevention and Control; 2012. [Online] Available from: http://www.unaids.org/sites/default/files/en/dataanalysis/knowyourresponse/countryprogressreports/2012countries/ce_VN_Narrative_Report.pdf [Accessed on 1st March, 2015]

[2] The Socialist Republic of Viet Nam. Declaration of Commitment on HIV and AIDS adopted at the 26th United Nations General Assembly Special Session in June 2001 (UNGASS). Hanoi: The Socialist Republic of Viet Nam; 2010. [Online] Available from: http://www.unaids.org/sites/default/files/en/dataanalysis/knowyourresponse/countryprogressreports/2010countries/vietnam_2010_country_progress_report_en.pdf [Accessed on 21st May, 2015]

[3] Ministry of Health. Guidelines for HIV/AIDS diagnosis and treatment. Hanoi: Ministry of Health; 2009. [Online] Available from: http://www.ilo.org/wcmsp5/groups/public/---ed_protect/---protrav/---ilo_aids/documents/legaldocument/wcms_177880.pdf [Accessed on 19th May, 2015]

[4] Pham QD, Wilson DP, Zhang L. A review of the extent of HIV drug resistance in Vietnam. *J AIDS Clin Res* 2012; doi: 10.4172/2155-6113.S5-001.

[5] Phan TT, Ishizaki A, Phung DC, Bi X, Oka S, Ichimura H. Characterization of HIV type 1 genotypes and drug resistance mutations among drug-naive HIV type 1-infected patients in Northern Vietnam. *AIDS Res Hum Retroviruses* 2010; **26**(2): 233-5.

[6] Ishizaki A, Cuong NH, Thuc PV, Trung NV, Saijoh K, Kageyama S, et al. Profile of HIV type 1 infection and genotypic resistance mutations to antiretroviral drugs in treatment-naive HIV type 1-infected individuals in Hai Phong, Vietnam. *AIDS Res Hum Retroviruses* 2009; **25**(2): 175-82.

[7] Tran VT, Ishizaki A, Nguyen CH, Hoang HT, Pham HV, Bi X, et al. No increase of drug-resistant HIV type 1 prevalence among drug-naive individuals in Northern Vietnam. *AIDS Res Hum Retroviruses* 2012; **28**(10): 1349-51.

[8] Nguyen TH, Duc NB, Shrivastava R, Tran TH, Nguyen TA, Thang PH, et al. HIV drug resistance threshold survey using specimens from voluntary counseling and testing sites in Hanoi, Vietnam. *Antivir Ther* 2008; **13**(Suppl 2): 115-21.

[9] Dean J, Ta Thi TH, Dunford L, Carr MJ, Nguyen LT, Coughlan S, et al. Prevalence of HIV type 1 antiretroviral drug resistance mutations in Vietnam: a multicenter study. *AIDS Res Hum Retroviruses* 2011; **27**(7): 797-801.

[10] Trinh QD, Pham NT, Le Nguyen NT, Lam BQ, Le Phan KT, Truong KH, et al. Short communication: drug resistance mutations in the HIV type 1 protease and reverse transcriptase genes in antiretroviral-naive Vietnamese children. *AIDS Res Hum Retroviruses* 2012; **28**(10): 1305-7.

[11] Stanford University, HIV Drug Resistance Database. Interactive map displaying HIV-1 drug resistance in ARV-naive population. Stanford: Stanford University, HIV Drug Resistance Database; 2015. [Online] Available from: http://hivdb.stanford.edu/ [Accessed on 27th March, 2015]

[12] Johnson VA, Calvez V, Gunthard HF, Paredes R, Pillay D, Shafer RW, et al. Update of the drug resistance mutations in HIV-1: March 2013. *Top Antivir Med* 2013; **21**(1): 6-14.

[13] Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antivir Ther* 2008; **13**(Suppl 2): 25-36.

[14] Pham HV, Ishizaki A, Nguyen L, Phan C, Phung T, Takemoto K, et al. Two-year outcome of first-line antiretroviral therapy among HIV-1 vertically infected children in Hanoi, Vietnam. *Int J STD AIDS* 2014; doi: 10.1177/0956462414556328.

[15] Phan CT, Pham HV, Bi X, Ishizaki A, Saina M, Phung CD, et al. Genetic analyses of HIV strains transmitted from mother to child in Northern Vietnam. *AIDS Res Hum Retroviruses* 2015; doi: 10.1089/aid.2014.0335.

[16] Chen M, Ma Y, Duan S, Xing H, Yao S, Su Y, et al. Genetic diversity and drug resistance among newly diagnosed and antiretroviral treatment-naive HIV-infected individuals in western Yunnan: a hot area of viral recombination in China. *BMC Infect Dis* 2012; **12**: 382.

[17] Kumar M, Sen S, Gupta RK, Chaturbhuj D, Tripathy SP, Paraniape RS. Prevalence of drug resistance in human immunodeficiency virus type 1-infected treatment-naive children in Pune, India. *AIDS Res Hum Retroviruses* 2014; **30**(3): 294-8.

[18] Sungkanuparph S, Manosuthi W, Kiertiburanakul S, Saekang N, Pairoj W, Chantratita W. Prevalence and risk factors for developing K65R mutations among HIV-1 infected patients who fail an initial regimen of fixed-dose combination of stavudine, lamivudine, and nevirapine. *J Clin Virol* 2008; **41**(4): 310-3.

[19] Bulgheroni E, Croce F, Citterio P, Viganò O, Visonà R, Sala E, et al. Unusual codon 69 insertions: influence on human immunodeficiency virus type 1 reverse transcriptase drug susceptibility. *J Clin Virol* 2004; **29**(1): 27-32.

[20] Nhung VTT, Colby D, Khanh TH, Viet TT, Lu D, Thuy HT, et al. HIV drug resistance in children with treatment failure to first-line regimens in Ho Chi Minh City, Vietnam. *J Int AIDS Soc* 2010; **13**(Suppl 4): P140.

[21] Giang LT, Thuy HT, Nhung VT, Liem NT, Khanh TH, Khanh LV, et al. ARV resistance in patients with treatment failure to first-line regimens in Ho Chi Minh city, Vietnam. Mexico: AIDS 2008 - XVII International AIDS Conference: Abstract no. TUPDA201; 2008.

[22] Shirasaka T, Kavlick MF, Ueno T, Gao WY, Kojima E, Alcaide ML, et al. Emergence of human immunodeficiency virus type 1 variants with resistance to multiple dideoxynucleosides in patients receiving therapy with dideoxynucleosides. *Proc Natl Acad Sci U S A* 1995; **92**(6): 2398-402.

[23] Stone C, Ait-Khaled M, Craig C, Griffin P, Tisdale M. Human immunodeficiency virus type 1 reverse transcriptase mutation selection during *in vitro* exposure to tenofovir alone or combined with abacavir or lamivudine. *Antimicrob Agents Chemother* 2004; **48**(4): 1413-5.

[24] Nouhin J, Madec Y, Ngo-Giang-Huong N, Ferradini L, Nerrienet E. Increased risk of Q151M and K65R mutations in patients failing stavudine-containing first-line antiretroviral therapy in Cambodia. *PLoS One* 2013; **8**(8): e73744.

[25] Kavlick MF, Wyvill K, Yarchoan R, Mitsuya H. Emergence of multi-dideoxynucleoside-resistant human immunodeficiency virus type 1 variants, viral sequence variation, and disease progression in patients receiving antiretroviral chemotherapy. *J Infect Dis* 1998; **177**(6): 1506-13.